THE MECHANISM OF THE PRODUCTION OF SUPPRESSION OF URINE IN BLACKWATER FEVER

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Suppression of urine is a by no means infrequent sequel to an attack of blackwater fever. Owing to the extreme gravity of this symptom—responsible as it is for a great proportion of the fatal cases—the mechanism of its production is a subject of considerable practical importance. It is unnecessary here to refer in detail to the work of previous authors, as a full account of the bibliography of the subject is given in the papers of Werner* and Barratt and Yorke†. The work of these authors and of de Haan‡ upon the condition of the kidneys of blackwater fever patients who have succumbed from suppression of urine has demonstrated the existence of granular material in the lumen of the renal tubules, and these workers infer that the suppression of urine is of mechanical origin, dependent upon the occlusion of the tubules by granular plugs. On the other hand, a nervous inhibition of glomerular secretion is regarded by A. Plehn§ as the essential factor in this condition. Again, nephritis has been mentioned by some writers as playing an important part in its production.

* 'Ueber die Nieren beim Schwarzwasserfieber,' Archiv. für Schiffs- und Tropenhygiene, 1907, Band XI, Bieheft 6.

† 'An investigation into the Mechanism of Production of Blackwater,' Annals of Tropical Medicine and Parasitology, 1909, Vol. III, No. 1.

‡ 'Die Nieren beim Schwarzwasserfieber,' Archiv. für Schiffs- und Tropenhygiene, 1905, S. 22.

§ 'Die Nieren beim Schwarzwasserfieber,' Archiv. für Schiffs- und Tropenhygiene, 1903, S. 270.

For many years it has been known that the injection of such poisons as glycerine, pyrogallic acid, toluylen-diamin and haemolytic serum frequently produces haemoglobinuria and sometimes leads to suppression of urine. It is doubtful whether the condition produced by such injections can be regarded as at all comparable with that occurring in blackwater fever, since it is highly improbable that the poisonous effects of the drugs in question are limited to the red blood cells.

Barratt and Yorke* observed that intravenous injection of an isotonic solution of haemoglobin made from the animal's own erythrocytes was quickly followed by the appearance of haemoglobinuria. The injections resulted in an increased flow of urine. Sections of the kidneys of these animals showed the presence of a certain amount of granular material in the lumen of some of the tubules. In none of the animals was there any tendency towards anuria.

Whether the occurrence of haemoglobin free in the blood plasma and its subsequent elimination by the kidneys is sufficient to produce suppression of urine through mechanical blocking of the renal tubules, or, whether a nephritis, or nervous inhibition of glomerular secretion, is to be regarded as the essential factor in the development of this condition in blackwater fever is a question, therefore, which has not, as yet, been definitely decided. It was with the object of throwing further light upon this point that our investigations were undertaken.

Our experiments were, with certain modifications, carried out along lines similar to those employed by Barratt and Yorke, i.e., the production of experimental haemoglobinuria in rabbits by the intravenous injection of solutions of homologous haemoglobin.

Technique. An isotonic solution of haemoglobin was made as follows:—A rabbit was bled from the external jugular vein and the blood collected in oxalated saline solution. The red blood cells, separated from the plasma by centrifugalisation, were washed three times in normal salt solution and then laked by the addition of distilled water. Sufficient sodium chloride was now added to render the solution isotonic and the precipitated stromata thrown down by means of the centrifuge. The clear solution of

* *Loc. cit.*

haemoglobin was then withdrawn. The percentage of haemoglobin present in the solution was estimated, in terms of human red blood cells, by means of a von Fleischl haemoglobinometer. As it was undesirable to bleed the experimental animal to an undue degree, the haemoglobin solution was prepared in part from the animal's own red blood cells, and in part from those of other rabbits. In order to facilitate the collecting of specimens of urine the bladder was opened supra-pubically, a cannula inserted and the wound closed by sutures. The rabbit was then placed in a specially constructed box which allowed of moderately free movement, but was sufficiently narrow to prevent the animal from turning round. A rubber tube attached to the glass cannula passed through a hole at the end of the box into a capsule in which the urine was collected. The solution of haemoglobin, warmed to 37° C., was now slowly injected into one of the veins of the ear. After an interval of two or three minutes to allow of the haemoglobin solution being thoroughly distributed throughout the circulation, a sample of blood was removed from the other ear and the degree of haemoglobinaemia estimated. The urine in the cannula was observed to be tinged with haemoglobin a few minutes after the injection. From time to time the urine was removed from the capsule, its volume measured and the amount of haemoglobinuria estimated.

Our earlier attempts to produce anuria by this means were invariably unsuccessful. Injection of the haemoglobin solution was followed by an increased flow of urine. This was probably to be explained by the diuretic action of the sodium chloride solution in which the haemoglobin was dissolved. After a time the diuresis subsided, but, as a rule, it lasted until the haemoglobinuria was distinctly decreasing in amount.

In later experiments we endeavoured to reproduce more closely the conditions obtaining in blackwater fever in man. In this disease the blood is anaemic and the patient is more or less collapsed. It appears probable that a decreased filtering force in the glomeruli, resulting from a low blood pressure, may play a not unimportant part in the production of anuria in these cases.

With this consideration in view we decided to reduce the blood pressure of our experimental rabbits, partly by keeping the animals

on as dry a diet as possible for twelve hours previous to the injection of haemoglobin, and also during the experiment, and partly by bleeding the animal to a moderate degree from the external jugular vein.

In order to obviate as far as possible the diuretic action of the sodium chloride, the strongest obtainable solution of haemoglobin was employed. Such solutions were prepared by dissolving the washed erythrocytes in the minimum quantity of distilled water necessary to produce complete laking.

As a result of these precautions we succeeded in producing suppression of urine in a number of animals.

Condition of the blood during experiment. Only a trace of haemoglobinaemia was detectable in the blood of the animals at the commencement of the experiment before injection of the haemoglobin solution. As a rule, bands of oxyhaemoglobin were just visible in a column 20 mm. high corresponding to an amount of haemoglobin equal to less than 0·1 per cent. The amount of haemoglobin injected into the circulation at one time varied from 1·5 to 13 grammes. Examination of the blood from time to time showed that the amount of haemoglobinaemia gradually decreased. The rate of disappearance was not constant, however, but occurred more rapidly at first and then gradually became slower, whilst the last traces of haemoglobin solution persisted for many hours. Moreover, it was occasionally observed that when a second or third injection of haemoglobin solution was made in the same animal after an interval of six to twelve hours a high degree of haemoglobinaemia persisted for a considerably longer period than that resulting from the first injection, even though a greater quantity of haemoglobin was introduced on this occasion than on the second. This point is well illustrated in Table 11, where, after the first injection the haemoglobinaemia within five hours fell from 12·6 to 2·8 per cent., whilst, after the second injection it only decreased from 9·7 to 5·25 per cent. during a period of nearly sixteen hours.

The significance of this observation is not very obvious. As only a comparatively small proportion (Table 1) of the haemoglobin circulating in the plasma is eliminated through the kidneys into the urine, the greater portion must be broken up in the

body. It has been shown by Tarchanoff* that the intravenous injection of solutions of haemoglobin in dogs is followed by a considerable increase in the formation of bile pigment. Moreover, the work of Simpson† has demonstrated that in those cases of malaria in which there is reason to believe that considerable destruction of red blood cells is occurring, the amount of faecal urobilin is greatly increased. It appears probable, therefore, that

Table 1.—Comparison of the amount of haemoglobin injected intravenously with that excreted in the urine.

No. of Experiment	Amount of haemoglobin injected intravenously g.	Amount of haemoglobin excreted in urine g.	Ratio of amount injected to amount excreted in urine	Remarks
1	3·4	0·72	1 : 5	Urine contained haemoglobin when animal died.
2	16·2	0·97	1 : 5	,,
3	29·4	5·3	1 : 6	
4	29·8	6·34	1 : 5	
5	16·0	1·45	1 : 11	
6	41·0	2·8	1 : 15	
7	23·7	1·15	1 : 21	Suppression of urine
8	28·2	2·66	1 : 11	,,
9	20·8	1·5	1 : 14	,,

a large proportion of the haemoglobin introduced into the circulation is got rid of by the liver, and that after a time the liver cells lose to a certain extent their capacity for eliminating from the blood stream large quantities of haemoglobin, and hence the rate of disappearance of haemoglobinaemia may be in the later stages much retarded.

* 'Ueber die Bildung von Gallenpigment aus Blutfarbstoff,' Pflüger's Archiv., Band IX, S. 53 und 329.

† 'On Haemoglobin Metabolism in Malarial Fever,' Roy. Soc. Proc., 1911, Vol. LXXXIII, p. 174.

In spite of the fact that in several of our experiments very large quantities (as much as 41 grammes) of haemoglobin were introduced into the circulation, we never observed any icteric condition of the skin or conjunctivae. On the other hand, the faeces were usually found to be semi-fluid and green after two or three injections of haemoglobin.

Owing to the fact that a certain amount of blood was withdrawn from the animal, and that this was replaced by haemoglobin solution, haemocrit records made during the experiments showed, as a rule, a progressive diminution of the volume of red blood cells as compared with that of the plasma. This decrease in the relative volume of the erythrocytes was, however, much more marked in the earlier experiments than in those in which the animal was kept on a dry diet and in which exceedingly strong solutions of haemoglobin were injected.

Condition of urine during experiment. The urine in the glass cannula was found to be tinged with haemoglobin five to ten minutes after the intravenous injection of the haemoglobin solution. Estimation of the percentage of haemoglobin passed in the urine showed that although a high degree of haemoglobinuria was quickly reached, nevertheless there existed no definite relationship betwen the concentration of haemoglobin in the urine and that present in the blood plasma, a very high percentage of haemoglobinuria being frequently observed after injection of comparatively small amounts of haemoglobin. As a rule, the maximum percentage of haemoglobinuria was not developed at once, but occurred some time (two to four hours) after the injection. A further point of interest is that after a second or third intravenous injection of haemoglobin the concentration of haemoglobin in the urine did not reach so high a level as that following upon the first injection, although in some instances the amount of haemoglobin circulating in the blood plasma was considerably higher.

It is clear that the percentage of haemoglobin present in the urine at any given time would of necessity depend upon several factors—firstly, the amount of free haemoglobin in the blood plasma; secondly, the rate at which it passed through the renal epithelium; and, thirdly, the amount of water which the kidneys secreted. When a comparison of the volume of urine passed and

the percentage of haemoglobinuria is made at regular intervals after the intravenous injection of haemoglobin solution, it is found that the latter varies to a certain extent inversely with the former, i.e., the more urine voided the lower the percentage of haemoglobin which it contains. Although the diuretic action of the sodium chloride solution is sufficient to account for the fact that the maximum degree of haemoglobinuria is not developed for some time after the injection, yet it affords no adequate explanation of why, after the second or third injection of haemoglobin, the percentage of haemoglobinuria is much lower than that following the first injection, even though in this case the blood plasma contained less free haemoglobin than in the former. It cannot depend upon the fact that the haemoglobin was diluted by a greater secretion of water, as, after the second or third injection, the amount of urine voided was distinctly less than earlier in the experiment. The only possible explanation is that either less haemoglobin was being excreted by the renal epithelium or that the haemoglobin was being held up in the renal tubules. This point will be returned to later in considering the microscopical appearances of the kidneys.

Although the first few specimens of urine examined after intravenous injection of haemoglobin solutions were perfectly clear without any deposit on centrifugalising, later specimens were turbid and contained a considerable quantity of solid material. Frequently, soft red masses of a gelatinous consistency closely resembling clots were passed. Microscopical examination of the deposit in the earlier stages showed that it consisted almost entirely of granular débris and casts. The clot-like masses themselves were composed of granular débris held together by mucoid material. The individual granules were at first of small size, although later in the experiment casts consisting of very large granules (3-4 μ) were frequently seen. They were of a brownish red colour, similar to that of red blood cells in urine, and were obviously derived from haemoglobin. In addition to this granular material there were also large flattened epithelial cells originating from some portion of the urinary tract below the kidneys.

Later, the character of the deposit changed considerably. In addition to the granular material large numbers of renal epithelial cells—isolated and also in the form of epithelial casts (Fig. 1)—

were found. Both the free cells and also those of the casts were frequently loaded with fine brown granules. Occasionally, a complete cast of the epithelium lining a portion of a renal tubule was seen in the form of an epithelial cylinder filled with granular material. On a few occasions red blood cells were found and, rarely, well marked red cell casts (Fig. 1).

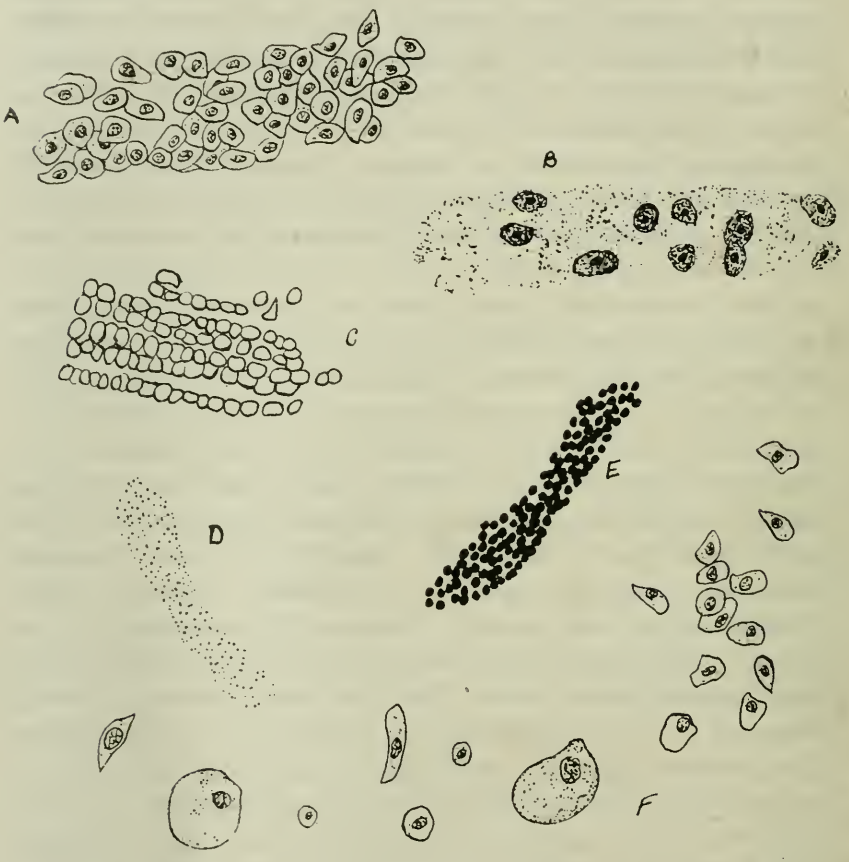

Fig. 1. Solid material passed in the urine of Rabbit 8 on the third day, during the period of almost complete suppression of urine. A—Epithelial cast. B—Finely granular cast in which are incorporated epithelial cells. C—Red blood cell cast. D—Finely granular cast. E—Cast consisting of coarse granules. F—Isolated epithelial cells. Magnification 300 diameters.

If no marked degree of suppression supervened, the urine cleared up after a certain time, depending upon the amount of haemoglobin injected, and the deposit of granular material and

epithelium gradually diminished, until, finally, the urine became normal. In several cases the flow of urine was greatly diminished or ceased altogether for a period. In Experiment 9 the animal developed complete anuria lasting over sixty hours; subsequently the secretion of urine gradually became re-established. In these cases (Rabbits 7, 8 and 9) the urine was greatly altered. No haemoglobinuria was observed. On acidifying with acetic acid and boiling, a dense white precipitate of albumen appeared. This was readily distinguishable from the dark chocolate precipitate which results when urine containing haemoglobin is boiled. On centrifugalisation there was a large deposit consisting of granular casts, some of which were built up of granules of very large size (3-4 μ), and enormous numbers of more or less degenerated renal epithelial cells and casts. This condition of the urine persisted until the animal's death.

Condition of the animal during the experiment.—Intravenous injection of large quantities of haemoglobin was in our experiments frequently followed by unfavourable symptoms. A considerable proportion of the rabbits injected died—some of them after even comparatively small doses (3·4 grammes). As a rule, the animal did not appear to be much affected during the first hour. Later, however, it became decidedly irritable, the slightest stimulus producing considerable reaction. Shortly before death the animal suddenly developed violent convulsions. Almost all the rabbits seemed to be more or less collapsed at first, but if they survived the injection longer than two hours they generally quickly recovered their normal condition. We were unable to discover the cause of death in those animals which died in convulsions. Careful post mortem examination conducted immediately upon the animal's death failed to reveal any signs of intravascular clotting either in the large veins, the heart, or in the pulmonary arteries.

Those animals which developed suppression of urine displayed characteristic symptoms. Chief among these were loss of appetite, rapid emaciation and considerable thirst (when once suppression of urine was established ordinary moist food was given). Death occurred in these animals two to eight days after the development of suppression of urine.

Condition of the kidneys. In considering the conditions

obtaining in the kidneys of these rabbits it is desirable to divide the experiments into two classes; firstly, those in which there was no indication of suppression of urine; and, secondly, those in which there was a more or less marked diminution in the amount of urine passed.

The kidneys of the first group varied considerably, both macroscopically and microscopically, according to the amount of haemoglobin solution injected and also according to the length of time which elapsed between the intravenous injection of

Table 2.—Weight of Kidneys in experimental and normal rabbits

No. of Experiment	Weight of rabbit, g.	No. of times haemoglobin solution was injected	Amount of haemoglobin solution injected g.	No. of hours animal lived after first injection of haemoglobin solution	Weight of Kidneys, G.		Remarks
					Right	Left	
I*	1,400	1	2·5	1·5	5·7	6·0	
II*	1,420	1	4·9	1·3	7·3	7·0	
[illegible]I	1,980	1	3·4	3·5	7·0	7·2	
III*	1,860	1	8·6	7	7·7	7·25	
IV*	1,570	2	11·3	22	6·8	6·5	
2	1,650	2	16·2	20	6·3	6·2	
3	1,750	4	29·4	38	6·7	6·8	
4	1,920	4	37·8	44	10·0	10·0	
5	2,870	5	16·3	98	10·5	10·5	
6	1,630	8	41·0	70	10·35	10·4	
7	1,235	7	23·7	68	11·55	10·75	Suppression of urine
8	1,740	3	28·2	94	11·0	10·75	
9	1,960	3	20·8	217	14·15	13·8	
Normal ...	1,550	—	—	—	6·0	6·1	
Normal ...	2,300	—	—	—	7·2	7·4	

haemoglobin and the removal of the kidneys. The kidneys of those animals which died after a single injection were but slightly altered in appearance. They were congested and of a dark brown or chocolate colour, but were not definitely enlarged (Table 2). Microscopically, a number of the convoluted tubules were seen to contain brownish coloured plugs. These were also present to a less extent in the tubules of the sub-cortical zone. There was no trace of solid material in the meniscus of Bowman's capsules nor in the collecting tubules of the papilla (ducts of Bellini). No dilatation of the tubules or glomeruli was observed.

* These experiments are not included in Tables 3-11.

A much more striking picture was afforded, however, by those kidneys which were removed after several injections of haemoglobin. Here these organs were distinctly enlarged and heavier than those of normal rabbits (Table 2). As before they were of a dark chocolate colour. On section the cortex was found to be thicker than normal, and the whole kidney was seen to be traversed by dark lines, giving it a markedly striated appearance. Frequently these dark coloured streaks were grouped in fan-shaped areas (Fig. 2), whilst the aspect of the intervening spaces was normal. The microscopical appearances presented by the kidneys were very characteristic. A large number of the convoluted tubules and tubes of Henle were filled with plugs of granular material, and here and there epithelial cells were found intermingled with the granular casts. The casts extended down into the large collecting tubules of the papilla. As before, no deposit was found in the meniscus of Bowman's capsules. Many of the convoluted tubules and tubes of Henle were distended and the epithelium in places had broken away from the basement membrane.

The kidneys of the animals belonging to the second group—those in which total or partial suppression of urine occurred—were profoundly changed. They were of a dark brown colour and greatly increased in size (Fig. 2) sometimes weighing more than twice as much as those of a normal rabbit (Table 2). On section they were found to be markedly oedematous. The cortical and sub-cortical zones were greatly thickened. Dark striations were visible, but they were not nearly so distinct as in the kidneys belonging to the previous group, and the appearance was confined chiefly to the sub-cortical portion of the pyramid. Microscopical examination showed that the whole structure of the kidney was radically altered. The tubules of the cortex and sub-cortical region were enormously dilated and many of them contained casts of granular material and epithelium in various stages of degeneration. The epithelium lining the tubules was in many places exceedingly thin, and in others had completely disappeared.

Scattered here and there between these large dilated tubules were small islands of tubules tightly packed together, so that their lumina were completely obliterated. Some of the glomeruli were enormously distended and others, like certain of the tubules, so

crushed as to be scarcely recognisable. The large collecting tubules were also much involved. In many places the epithelial cells had broken away from the basement membrane and were either lying

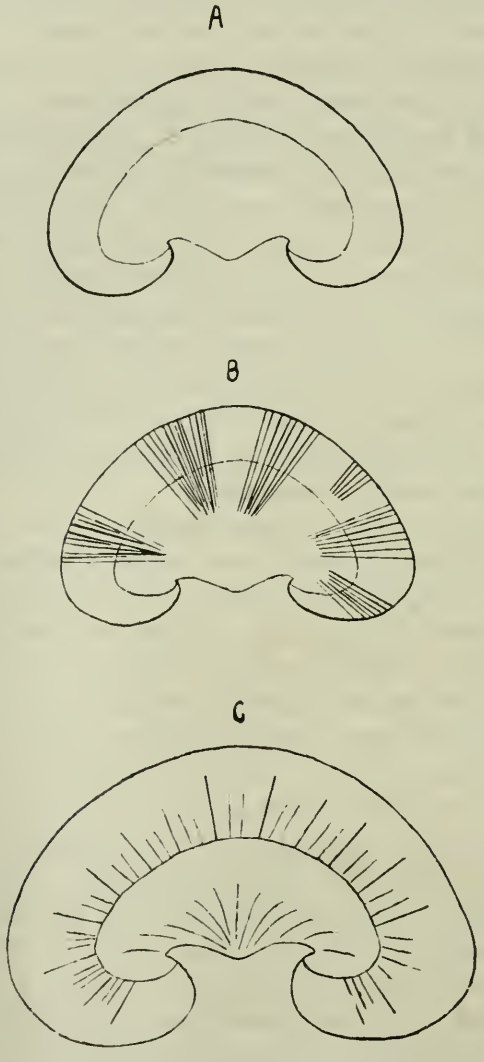

Fig. 2. Diagrammatic representation of the appearances found on section of—A, normal kidney; B, kidney of an animal which had received several injections of haemoglobin; C, kidney of Rabbit 9, in which there was complete anuria for 60 hours. Magnification 1·5 diameters.

loose within the tubule or had entirely disappeared. In other tubules the epithelium was intact, but the lumina were filled with

masses of epithelial cells and granular débris. Occasionally, a collecting tubule was found in which the lining epithelium was normal and the lumen entirely filled by a mass of granular material and isolated epithelial cells surrounded by a ring of epithelium evidently derived from a higher portion of the same collecting tubule.

As to the exact nature of the granular casts existing in the renal tubules and subsequently voided in the urine, it is difficult to speak with certainty. When examined in the fresh unstained condition their colour resembled closely that of the red blood cells. Moreover, they stain with various dyes (eosin, van Gieson, iron alum haematoxylin) in a manner precisely similar to the erythrocytes. There appears to be no doubt but that they are derived from haemoglobin, and we are of opinion that these casts are composed of minute droplets of haemoglobin (or of a very closely allied substance) held together by mucoid material. Of course this statement refers only to the casts existing in the renal tubules and urine examined within a few hours of the injection. The casts examined at a later period of time (four to eight days) after the haemoglobin injection, were obviously different. The material had in places changed to a light brown colour and no longer stained well. In other places it had become in part crystalline. We were unable to obtain the iron reaction with ammonium sulphide and ferrocyanide of potassium, probably because the decomposition of the haemoglobin had not progressed sufficiently far. This reaction was obtained by Werner in several cases of blackwater fever in man.

We have hitherto avoided any reference to the question as to which portion of the renal tubule is responsible for the elimination of haemoglobin. As we intend to discuss this question more fully in a future communication, we shall content ourselves here with stating that as a result of our observations we are of opinion that the haemoglobin does not pass through the glomeruli, but that it is excreted by the epithelium of the renal tubules, more especially by that of the convoluted tubules.

If the information obtained from examination of the urine be considered in conjunction with that derived from a study of the pathological condition obtaining in the kidneys, one is enabled to

form a fairly definite conception as to the effect which intravenous injection of haemoglobin has upon the renal secretion of an otherwise normal animal. The immediate result of such injections is a diuresis, dependent upon the action of the sodium chloride solution used for dissolving the haemoglobin. At the same time the renal epithelium commences to excrete haemoglobin into the tubules. At first this is quickly carried away by the rapid secretion of watery urine from the glomeruli. The result is a considerable flow of clear urine containing haemoglobin in solution. As the diuresis subsides the haemoglobin becomes deposited to a certain extent in the tubules. At this stage the urine is less in amount and contains a quantity of solid material consisting chiefly of granular casts and débris. Still later, especially when the degree of haemoglobinaemia is high, epithelial cells are shed from the lining membrane of the renal tubules. This is probably dependent upon one or both of the following factors. In the first place it is possible that the excretion of large quantities of a foreign substance like haemoglobin may be attended by injurious effects upon the renal epithelium. In the second place the presence of casts in the lumen of the tubules would tend to damage the lining epithelium mechanically, either by friction as the plug was forced down the tubule owing to the *vis a tergo* of the glomerular secretion or by causing a complete or partial block of the tubule, and, as a result, dilatation of that portion above the plug, and consequently separation of the epithelium from the basement membrane (Plate XIII, figs. 3 and 4). This stage represents the commencement of the development of suppression of urine. Gradually, as haemoglobin continues to be excreted by the renal epithelium more and more tubules become occluded. The situation in which complete blocking of the lumen first occurs appears to be, as a rule, the narrow tubules of Henle. Coincidently with the plugging of the tubules there is a marked diminution in the amount of urine voided and also of the haemoglobin it contains. The condition steadily progresses, until, finally, complete anuria (Case 9) results. After cessation of the flow of urine has lasted for a certain time a gradual re-establishment of urinary secretion occurs. The urine is loaded with albumen and is exceedingly turbid. On centrifugalisation a large deposit, sometimes equalling as much as

one-fourth of the volume of urine, is thrown down. This deposit consists of granular casts, epithelial casts, granular débris and degenerated epithelial cells. Urine of this nature continues to be passed until the animal's death. During the period of total or partial anuria marked changes occur in the kidneys. Owing to the continued secretion of the glomeruli those portions of the tubules above the plugs become enormously distended with fluid. As a result, the epithelium lining these portions becomes extremely thin and in places entirely disappears. The dilated tubules press upon adjacent tubules, so that on microscopic examination the latter appear of small size and their lumina are completely obliterated (Plate XII, fig. 1). After a time, owing to gradual disintegration of the casts plugging the tubes, the urine commences to escape into the ureter once more. The serum proteids and red blood cells pass through the damaged epithelium and those portions of the tubules devoid of epithelial cells, with the result that the urine is loaded with albumen. Although the casts may be to a great extent washed away in the course of the next few days, yet the renal tubules remain dilated and the serum proteids continue to escape into the urine until the animal's death, which in these cases is probably due to uraemia.

CONCLUSIONS

It is evident from these experiments that, under certain conditions, the mere passage of haemoglobin through the kidneys of a healthy animal is sufficient to cause suppression of urine owing to occlusion of the lumen of the renal tubules by plugs of granular material derived from the haemoglobin. The process is considerably facilitated by any factor which tends to lower the blood pressure of the animal, and as a result the secretion of water by the malpighian capsules. On the other hand, when the blood volume of the animal is kept up, as in Case 4, by the injection of saline solution (that of the haemoglobin solution which in this case was not particularly strong) and by feeding the animal on moist food, a large amount of haemoglobin (38 grammes) may be injected without any tendency to suppression of urine.

Table 3. Rabbit 1, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
1st ...	1 p.m.	1,980	22	...	...	38·0	...	...	
	2.35	...	...	3·4	...	...	...	...	
	2.45	...	...	...	6·5	...	...	...	
	2.55	...	...	...	...	...	4·4	3·4	
	3.15	...	...	...	...	...	5·5	2·1	
	3.30	...	...	...	...	...	4·5	1·7	
	3.45	...	...	...	...	...	5·0	1·0	
	4.45	...	...	...	...	...	4·7	4·0	
	5.25	...	...	...	...	...	1·0	3·9	
	5.45	...	...	...	...	...	1·25	3·6	
	6	...	...	...	1·4	...	1·2	4·0	Animal died with slight convulsions

Table 4. Rabbit 2, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
1st ...	9 p.m.	1,650	...	...	Trace	40·5	...	...	
	10	...	...	4·0	...	...	...	...	
	10.10	...	...	...	6·0	...	...	...	
2nd ...	12.15 a.m.	...	...	...	...	...	8·0	3·7	
	7	...	...	...	...	...	28·5	2·3	
	10	...	...	...	...	...	14·5	0·0	
	10.25	1,535	...	...	...	...	...	...	
	10.35	...	19	...	0·37	47·0	...	...	
	11.10	...	...	12·2	...	...	...	...	
	1 p.m.	...	...	...	...	...	10·0	4·6	
	3	...	...	...	...	...	4·5	15·7	
	5	...	...	...	5·4	34·0	12·0	7·0	
	5.25	...	15	...	...	...	...	...	
	5.45	...	...	...	...	...	...	...	Animal collapsed
	6	...	...	...	...	...	...	...	Death

Table 5. Rabbit 3, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobinaemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobinuria	
1st ...	11.10 a.m.	1,750	...	3·4	...	...	...	...	
	11.15	...	...	...	4·8	...	...	...	
	12.45 p.m.	...	...	...	...	...	2·6	2·5	
	3.30	...	...	...	...	...	8·0	5·1	
	5	...	18	...	0·34	43·5	5·0	0·5	
	5.30	...	...	9·6	13·0	...	...	...	
	7	...	...	...	...	...	10·0	3·2	
	10	...	...	...	...	...	11·5	7·2	
	11.30	...	16	...	2·4	35·5	5·5	3·4	
	12.25 a.m.	...	...	11·9	18·0*	...	...	...	* Calculated
2nd ...	1.20	...	...	...	...	...	19·0	3·7	
	7.40	...	...	...	...	...	27·5	5·5	
	10.30	...	...	...	...	...	16·0	2·0	
	11.45	...	11	...	1·8	30·0	...	...	
	12.10 p.m.	...	...	...	...	...	4·5	1·5	
	12.15	...	...	4·5	...	...	...	...	
	1	1,63[illegible]	...	...	...	...	4·5	1·8	
	2.3[illegible]	...	...	...	...	...	8·5	5·5	
	4.15	...	...	...	...	...	10·0	3·3	
	6.3[illegible]	...	15	...	1·7	25·0	9·5	2·9	
	7.45	...	...	...	...	...	1·0	2·2	Animal died suddenly

TABLE 6. Rabbit 4, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobinaemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobinuria	
1st ...	6 p.m.	1,920	30	...	Trace	39·0	...	...	
2nd ...	11 a.m.	...	...	...	...	...	17·0	0·0	
	3.30	...	...	...	...	...	10·0	0·0	
	4.20	...	...	9·2	...	...	...	...	
	4.40	...	...	...	10·0	...	3·0	2·7	
	5.5	...	...	...	...	...	3·8	5·1	
	6.20	...	...	...	...	...	4·5	17·8	
	8.40	...	...	...	...	...	3·4	16·6	
	10.20	...	...	...	...	...	0·6	10·0	
	11.45	...	...	...	...	...	7·0	4·6	
3rd ...	12.5 a.m.	...	18	...	1·2	24·5	...	...	
	12.20	...	...	7·6	...	...	...	...	
	12.25	...	...	...	9·5	...	0·0	...	
	4	...	...	...	...	...	2·0	13·5	
	11	...	...	...	...	...	10·0	4·0	
	4 p.m.	...	...	...	...	...	3·5	0·0	
	4.30	...	...	...	...	...	2·0	0·0	
	4.50	...	22	...	0·3	23·7	...	...	
	5.40	...	...	13·0	...	...	...	...	
	6.50	...	...	...	...	...	12·0	4·8	
	10	...	...	...	...	...	...	...	
	10.45	...	...	...	...	...	18·0	7·3	
4th ...	8 a.m.	...	...	...	...	...	2·0	12·1	
					...	...	20·0	7·3	
	11.20	...	...	...	...	...	7·0	1·3	
	12.10	1,640	...	8·0	...	...	3·0	0·2	Animal died immediately after the injection

TABLE 7. Rabbit 5, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
1st ...	11.30 a.m.	2,870	26	...	...	33·0	...	...	
	3 p.m.	...	...	3·65	...	...	...	...	
	3.10	...	...	...	4·2	...	...	...	
	3.45	...	...	...	...	...	0·75	2·7	
	4	...	...	...	2·9	...	...	...	
	4.45	...	...	...	...	...	2·6	6·8	
	5.45	...	...	...	...	...	3·8	5·1	
	6.10	...	...	...	...	...	1·8	2·5	
	6.45	...	...	...	...	...	3·0	3·0	
	8	...	...	...	...	...	5·0	0·45	
	8.15	...	...	...	0·4	30·0	...	...	
	8.45	...	30	...	...	...	...	...	
	10.45	...	...	3·4	...	...	...	...	
	11	...	...	...	4·5	...	...	...	
2nd ...	10 a.m.	...	...	...	...	...	11·0	2·0	
	10.30	...	...	...	...	...	1·0	0·2	
	11	...	...	...	0·3	26·4	...	...	
	11.30	...	37	...	...	...	...	...	
	1.45 p.m.	...	...	4·0	...	...	...	...	
	1.50	...	...	...	5·0*	...	...	...	* Calculated
	5.30	...	...	...	...	...	3·0	5·8	
	6	...	...	...	...	...	1·0	1·5	
	10.45	...	34	...	0·15	21·0	13·0	0·2	
3rd ...	12.10 a.m.	...	...	3·5	...	...	...	...	
	12.20	...	...	...	4·5*	...	...	...	* Calculated
	9	...	...	...	...	...	38·0	0·5	
	11	...	...	...	...	...	10·0	0·0	
	11.15	...	25	...	...	14·0	...	...	
	1.45 p.m.	...	...	1·8	...	...	...	...	
	1.50	...	...	...	1·5	...	...	...	
	2.15	...	...	...	...	...	3·0	1·5	
	4	...	...	...	...	...	2·7	6·4	
	5	...	...	...	...	...	4·8	1·5	
	11.30	...	...	...	...	...	17·0	0·2	
4th ...	10 a.m.	...	...	...	...	...	33·0	0·0	
	11 p.m.	...	...	...	...	...	24·0	0·0	
5th ...	10 a.m.	...	...	...	...	...	20·0	0·0	
	5 p.m.	...	...	...	...	...	7·0	0·0	Animal died

Table 8. Rabbit 6, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
1st ...	12.20 p.m.	1,630	18	...	Trace	42·5	...	...	
	1.18	...	...	3·0	...	...	...	...	
	1.23	...	...	...	4·7	...	...	...	
	2.50	...	...	...	...	...	1·9	6·3	
	4	...	...	...	...	...	1·8	10·33	
	5	...	...	...	...	...	1·3	17·9	
	5.45	...	...	...	...	...	1·4	5·0	
	6.10	...	...	4·4	...	...	2·2	3·0	
	6.14	...	...	...	5·8	...	...	...	
	9	...	...	...	...	...	2·0	7·2	
	11.30	...	16	...	1·3	33·0	2·5	21·0	
2nd ...	12.5 a.m.	...	...	4·7	...	...	...	...	
	12.15	...	...	...	7·0*	...	...	...	* Calculated
	10	...	...	...	...	...	7·0	8·3	
	11.50	1,580	...	...	...	...	2·5	1·5	
	12.5 p.m.	...	...	6·0	...	...	...	...	
	12.10	...	...	...	9·0	...	...	...	
	3	...	...	...	...	...	4·5	4·8	
	4.30	...	...	...	...	...	2·0	4·8	
	6.30	...	...	...	...	...	3·7	3·45	
	8.55	...	11	...	1·5	29·6	...	...	
	10	...	...	5·4	...	...	...	...	
	10.15	...	...	...	9·0*	...	...	...	* Calculated
3rd ...	12.5	...	...	...	...	...	2·5	3·65	
	7.10	...	...	...	...	...	10·0	2·2	
	10.15	...	...	...	...	...	4·75	0·7	
	10.25	...	20	...	1·2	22·0	...	...	
	11	...	...	6	...	...	...	...	
	11.5	...	...	...	9·5*	...	...	...	* Calculated
	1 p.m.	...	...	...	...	...	1·5	2·7	
	3	...	...	...	...	...	4·0	5·0	
	5.30	...	...	...	...	...	3·75	4·25	
	5.45	...	...	7	...	...	...	...	
	5.50	...	...	...	10·5	...	...	...	
	8.25	1,540	...	...	...	...	...	...	
	8.30	...	...	...	...	...	1·7	3·7	
	11.30	...	12	...	5·45	15·5	2·8	3·18	
	11.50	...	...	4·45	...	...	...	...	
4th ...	12.50 a.m.	...	...	...	...	...	0·0	...	
	7.10	...	...	...	...	...	0·8	3·0	
	10.30	...	...	...	...	...	0·7	2·4	Animal died

Table 9. Rabbit 7, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
1st ...	3.45 p.m.	1,235	...	1·5	...	...	...	...	
	4.30	...	...	...	...	...	3·0	4·2	
	5.45	...	14	...	1·3	35·0	2·0	2·3	
	5.54	...	...	3·0	...	...	...	...	
	6.30	...	...	...	...	...	5·0	4·0	
	7	...	...	...	...	...	1·0	8·0	
	9.30	...	18	...	1·0	23·75	2·0	2·0	
	10.20	...	...	4·6	...	...	...	...	
2nd ...	1.15 a.m.	...	...	...	...	...	7·0	2·7	
	1.35	...	...	3·9	...	...	...	...	
	7.15	...	...	...	...	...	16·5	1·9	
	10.30	...	...	...	...	...	9·0	1·6	
	1.30 p.m.	1,100	...	...	...	...	...	...	
	2.40	...	...	3·0	...	...	0·5	2·2	
	7	...	...	...	...	...	0·75	1·8	
	9	...	...	...	...	...	0·25	1·8	
	11.30	...	...	...	...	...	0·8	3·6	
	11.35	...	...	3·3	...	...	...	...	
3rd ...	12.5 a.m.	...	...	...	...	...	0·0	...	
	1.30	...	...	...	...	...	0·3	4·0	
	7.30	...	...	...	...	...	0·5	1·5	
	11.30	...	...	...	...	...	0·5	1·5	
	12.5 p.m.	1,120	...	...	...	...	...	...	
	12.40	...	...	4·4	...	...	...	...	
	5	...	...	...	...	...	0·9	2·0	
	11	...	...	...	...	...	1·5	0·9	
4th ...	12.5 a.m.	...	...	...	...	...	0·0	...	
	9	...	...	...	...	...	3·0	0·4	
	11	...	...	...	...	...	...	...	Animal died

Table 10. Rabbit 8, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobinaemia	Haemocrit	Amount of urine passed c.cm. †	Percentage of haemoglobinuria	
1st ...	10 p.m.	1,740	...	...	...	...	...	...	
	10.45	...	...	4·3	...	...	...	...	
2nd ...	7 a.m.	...	...	...	...	...	32·0	1·2	
	10.30	...	...	...	...	...	18·0	0·2	
	10.35	1,630	...	...	...	...	...	...	
	10.45	...	20	...	0·4	47·5	...	...	
	11.55	...	...	12·9	...	...	...	...	
	12.5 p.m.	...	...	...	16·9	...	...	...	
	1	...	...	...	...	...	8·0	3·1	
	3	...	...	...	...	...	5·0	5·0	
	5	...	...	...	...	...	16·0	6·7	
	7	...	...	...	...	...	2·0	8·9	
	9.15	...	...	...	...	...	3·0	5·0	
	10.5	...	...	...	...	...	0·6	4·1	
	11.35	...	...	...	...	...	1·4	3·0	
	11.50	...	...	...	2·2	42·0	...	...	
3rd ...	12.45 a.m.	...	...	11	...	...	...	...	
	12.50	...	...	...	15·5*	...	...	...	* Calculated
	2	...	...	...	...	...	0·0	...	
	7	...	...	...	...	...	3·4	4·8	
	12.5 p.m.	1,600	...	...	...	...	0·7	5·0	
	12.50	...	...	...	...	...	0·7	4·6	
	1	...	...	...	...	...	0·0	...	
	11	...	...	...	...	...	0·9	2·9	
4th ...	1 a.m.	...	...	...	...	...	0·0	...	
	9	...	...	...	...	...	4·0	0·75	
	1 p.m.	1,575	...	...	0·3	45·0	1·3	0·0	Heavy white precipitate of albumen on acidifying with acetic acid and boiling. Considerable deposit of granular casts and epithelial cells
	6	...	...	...	...	...	2·2	0·0	
	11.55	...	...	...	...	...	2·0	0·0	
5th ...	1 a.m.	...	...	...	...	...	0·0	...	
	3	...	...	...	...	...	0·8	0·0	
	7	...	...	...	...	...	0·9	0·0	
	8	...	...	...	...	...	0·0	...	
	8.30	1,500	...	...	...	...	0·0	...	Animal died

Table 11. Rabbit 9, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
	10 a.m.	1,960	...	...	...	...	...	...	
1st ...	11	...	30	...	Trace	29·5	...	...	
	3 p.m.	...	...	10·8	...	...	...	...	
	3·5	...	...	...	12·6	...	...	...	
	4.15	...	...	...	...	...	2·0	8·0	
	5	...	...	...	...	...	1·7	11·4	
	5.15	...	...	...	...	...	2·7	9·75	
	8	...	...	...	...	...	11·0	4·5	
	9	...	16	...	2·8	20·4	9·0	3·0	
	10.30	...	...	...	...	...	1·5	1·6	
2nd ...	12.35 a.m.	...	...	7·5	...	...	...	...	
	12.40	...	...	...	9·7	15·5	0·9	3·2	
	1.30	...	...	...	...	...	0·0	...	
	7·30	...	...	...	...	...	7·1	1·1	
	10.30	1,850	...	...	...	...	0·0	...	
	1 p.m.	...	...	...	...	...	0·0	...	
	4	...	...	...	5·25	...	0·0	...	
	8.45	...	...	2·5	...	...	...	...	
	9.15	...	...	...	...	...	0·0	...	
	11.30	...	...	...	...	...	0·0	...	
3rd ...	10 a.m.	1,850	...	...	...	...	0·0	...	
	11.30	...	...	...	...	...	0·0		
	5 p.m.	...	...	...	...	18·5	0·0	...	
	11.30	...	...	...	...	...	0·0	...	
4th ...	9 a.m.	1,840	...	...	...	...	0·0	...	
	1 p.m.	...	...	...	...	...	0·6	0·0	
	3	...	...	...	Trace	18·5	0·0	...	
	5	...	...	...	...	...	1·5	0·0	
	10	...	...	...	...	...	1·0	0·0	
5th ...	8 a.m.	...	...	...	...	...	2·0	0·0	
	1 p.m.	...	...	...	...	...	1·6	0·0	
	10	...	...	...	...	...	7·3	0·0	Heavy white precipitate of albumen on acidifying with acetic acid and boiling
6th ...	12.5 a.m.	...	...	...	...	...	1·4	0·0	
	8	...	...	...	...	...	28·0	0·0	
	10	1,750	...	...	...	...	5·0	0·0	
	3 p.m.	...	...	...	...	...	2·0	0·0	
	6.30	...	...	...	...	...	2·5	0·0	
7th ...	12.5 a.m.	...	...	...	...	...	5·0	0·0	Considerable deposit of granular casts and renal epithelial cells
	10	1,640	...	...	...	...	12·0	0·0	
	7 p.m.	...	...	...	...	...	5·0	0·0	
	11.30	...	...	...	...	...	4·0	0·0	
8th ...	10 a.m.	...	...	...	...	...	13·0	0·0	
	5 p.m.	...	...	...	...	...	6·0	0·0	
9th ...	10 a.m.	1,460	...	...	...	...	10·0	0·0	
	5 p.m.	...	...	...	...	...	10·0	0·0	
10th ...	10 a.m.	1,390	...	...	...	...	2·0	0·0	
	4 p.m.	...	...	...	...	...	0·0	0·0	Animal died with slight convulsions

DESCRIPTION OF PLATES

The sections were stained by Heidenhain's iron alum haematoxylin method and the figures drawn with an Abbé camera lucida.

Plate XII

Fig. 1.—Section of renal cortex of Rabbit 9. Many of the convoluted tubules are greatly distended and in some the lining epithelium is considerably flattened. Scattered between the dilated tubes are islands of tubules tightly packed together, so that their lumina are completely obliterated. Two Bowman's capsules are seen in the section. A number of the dilated tubules contain plugs of granular material. Magnification 240 diameters.

Fig. 2.—Transverse section of renal pyramid (sub-cortical portion) of Rabbit 6, showing many dilated tubules (tubes of Henle and collecting tubules) completely plugged with granular material. Magnification 350 diameters.

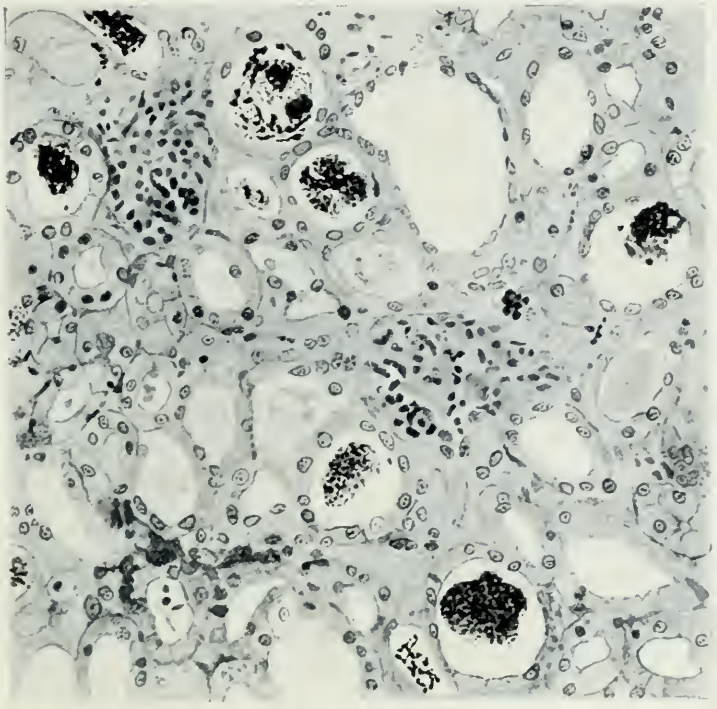

FIG. 1.

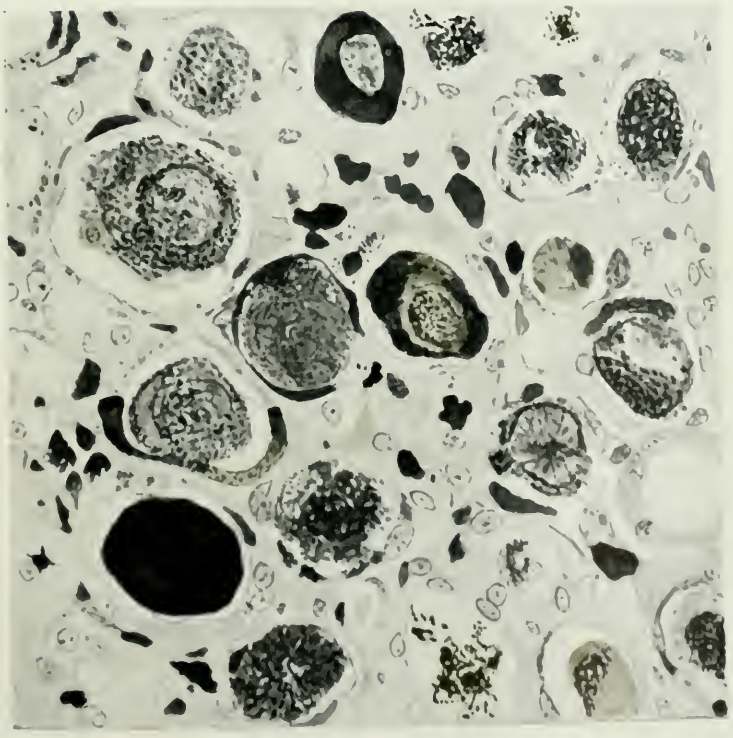

FIG. 2.

A. M. B., del. P. P. Press, Imp.